Magyarország-Szerbia
IPA Határon Átnyúló Együttműködési Program

Jó szomszédok a közös jövőért

# Környezetszennyező anyagok: problémák és lehetséges megoldások

# ABSZTRAKTKÖNYV

# LACREMED WORKSHOP

**2012. augusztus 31. – szeptember 1.**
**Szegedi Tudományegyetem, Természettudományi és Informatikai Kar, Mikrobiológiai Tanszék**
**Szeged, Magyarország**

A projekt az Európai Unió társfinanszírozásával valósul meg

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# Zagađujuća jedinjenja u životnoj sredini: problemi i moguća rešenja

# KNJIGA IZVODA

# LACREMED RADIONICA

**31. avgust – 1. septembar 2012**
**Departman za mikrobiologiju, Fakultet prirodnih nauka, Univerzitet u Segedinu**
**Segedin, Mađarska**

Projekat sufinansira Evropska unija

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**Prof. Dr. Biljana Škrbić**, Faculty of Technology, University of Novi Sad, Serbia
**Dr. Tamás Papp**, Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Hungary
**Dr. Judit Krisch**, Institute of Food Engineering, Faculty of Engineering, University of Szeged, Hungary

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**Dr. Judit Krisch**, Institut za prehrambeni inženjering, Inženjerski fakultet, Univerzitet u Segedinu, Segedin, Mađarska

# Contents

**Development of an enzymological (laccase-based) remediation product and technology**

**Lead beneficiary:** Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Hungary
**Project partner:** Faculty of Technology, University of Novi Sad, Serbia

**Project implementation period:** 01.01.2012– 31.12.2013.

## Background

Laccases are cuproproteins, so called blue oxidases that catalyze the oxidation of several aromatic and inorganic substances. These enzymes are able to oxidize and polymerize the xenobiotics into less soluble, high molecular mass compounds that may be easily removed from water by sedimentation or filtration; in soils, enzymes detoxify the pollutants by coupling them onto humic acids, immobilizing them and preventing their leaching. Many filamentous fungi produce extracellular laccases on cheap substrates. The well known laccase producers are the mushrooms *Agaricus* and *Pleurotus*, and some soil inhabiting moulds e.g. *Cladosporium*, *Alternaria* and *Botrytis*.
The microbial degradation of the permitted and widely used anilinogenic and phenoligenic pesticides results in chlorinated, highly toxic aniline and phenol derivatives. They could be found regularly in surface waters and agricultural soils. They have high ecotoxicological effects and also are dangerous to humans as some of them have immuno-modulating, endocrine-disrupting, mutagenic and carcino-genic effects.

## Objectives of the project

The aim of the project is the development of a cheap microbial laccase mixture, which is able to detoxify a wide range of xenobiotics belonging to the groups of phenol and aniline derivatives.

1. Isolation of distinct laccase producing filamentous fungi from soil and air on specific detection-isolation media.
2. Investigating of culture media dependence of laccase producing activity of the best strains.
3. Identification of the best laccase producers.
4. Investigation and determination of the substrate spectra of the distinct fungal laccases.
5. Investigation of activity levels of diluted laccase containing ferment broths on distinct pollutants.
6. Investigation of the stability ("half life times") of laccases in distinct water and soil types.
7. Comparative investigations in laboratory of detoxifying activity of fungal laccases and their mixtures on distinct phenol and aniline derivatives in water and soil microcosms systems.
8. In field investigations of the activity of laccases on 2,4-dichlorophenol and 3,4-dichloroaniline in the soils of distinct plant cultures.

# LACREMED projekt

## Enzim- (lakkáz-) alapú bioremediációs termék és technológia kifejlesztése

**Projektvezető:** Szegedi Tudományegyetem, Természettudományi és Informatikai Kar, Mikrobiológiai Tanszék
**Projekt partner:** Újvidéki Egyetem, Technológiai Kar

**Projektkivitelezési időszak:** 2012.01. 01. – 2013.12. 31.

## Háttér

A lakkázok réztartalmú fehérjék, úgynevezett kék enzimek, melyek számos aromás és szervetlen vegyület oxidációját katalizálják. Ezek az enzimek képesek a xenobitikumokat rosszul oldódó, nagy molekulatömegű vegyületekké alakítani, melyek ülepítéssel vagy szűréssel könnyen eltávolíthatók a vízből. A talajban az enzimek humuszsavakhoz kötik a szennyezőanyagokat, így meggátolják továbbterjedésüket, illetve kimosódásukat a talajból. Sok fonalasgomba képes lakkáztermelésre olcsó szubsztrátumokon. A legismertebb lakkáztermelők az *Agaricus* (csiperke) és *Pleurotus* (laskagomba) ehető gombák, valamint a talajban élő *Cladosporium*, *Alternaria* és *Botrytis* penészgombák.
Az Európai Unióban engedélyezett és gyakran alkalmazott anilin- és fenol-jellegű vegyületeket tartalmazó peszticidek mikrobiális lebontása klórozott, erősen toxikus származékokat eredményez. Ezek a származékok mérgezőek a környezetre nézve és veszélyesek az emberi szervezetre, mivel immunrendszert gyengítő, hormonműködést befolyásoló és rákkeltő hatásuk van.

## A projekt céljai

A projekt célja egy olyan lakkáz-enzimkeverék létrehozása, amely képes az anilin- és fenolszármazékok semlegesítésére.

1. Speciális tápközegek segítségével lakkáztermelő gombák izolálása talaj- és levegőmintákból.
2. A legjobb enzimtermelő törzsek tápközegtől függő lakkáztermelésének vizsgálata.
3. A legjobb lakkáztermelők fajszintű azonosítása.
4. Az egyes lakkáz enzimek szubsztrátspecifitásának vizsgálata.
5. A tápközegbe kiválasztott, nem tisztított lakkázok egyes szennyezőanyagokra gyakorolt hatásának vizsgálata.
6. A lakkázok stabilitásának (fél-életidő) vizsgálata víz- és talajmintákban.
7. Az egyes gombaeredetű lakkázok és keverékeik szerepének laboratóriumi összehasonlító vizsgálata anilin- és fenolszármazékok semlegesítésére víz- és talaj-mikroközösségekben.
8. Szántóföldi kísérletek a lakkázok 2,4-diklorofenol és 3,4-diklóramin elleni aktivitásának vizsgálatára növénykultúrákban.

# LACREMED projekta

Razvoj proizvoda i tehnologije za remedijaciju na bazi primene enzima (lakaze)

**Glavni korisnik:** Departman za mikrobiologiju, Fakultet prirodnih nauka, Univerzitet u Segedinu, Segedin
**Projektni partner:** Tehnološki fakultet, Univerzitet u Novom Sadu

**Period trajanja projekta:** 01. 01. 2012. – 31. 12. 2013.

## Pozadina

Lakaze su enzimi iz grupe metaloproteina sa bakrom, poznati i pod nazivom plave oksidaze, koji katalizuju oksidaciju različitih aromatičnih i neorganskih jedinjenja. Pod dejstvom ovih enzima dolazi do oksidacije i polimerizacije ksenobiotika, i njihovog prevođenja u slabo rastvorljiva jedinjenja velikih molekulskih masa, koja se sedimentacijom ili filtracijom mogu lako ukloniti iz vode; u zemljištu, lakaze utiču na imobilizaciju molekula zagađujućih jedinjenja vezivanjem sa huminskim kiselinama, smanjujući tako njihovu prvobitnu toksičnost i mobilnost. Mnoge filamentozne plesni razvojem na jeftinim i dostupnim supstratima stvaraju vanćelijske lakaze; najpoznatije su gljive *Agaricus* i *Pleurotus,* kao i neke plesni u zemljištu, kao na primer, *Cladosporium*, *Alternaria* i *Botrytis.*
Mikrobiološka degradacija pesticida na bazi anilinskih i fenolnih jedinjenja, čija upotreba je dozvoljena i široko rasprostranjena, dovodi do stvaranja hlorovanih derivata anilina i fenola, koja se mogu naći u površinskim vodama i obradivom zemljištu. Nastala jedinjenja imaju štetno dejstvo po zdravlje ljudi, zbog svoje kancerogenosti, mutagenosti, imunomodulatornog efekta i dejstva na endokrine žlezde, izazivajući takođe i značajne ekotoskikološke efekte.

## Ciljevi projekta

Cilj projekta je razvoj lako dostupnih smesa lakaza, sposobnih za razgradnju i detoksikaciju širokog spektra ksenobiotika iz grupe fenolnih i anilinskih derivata.

1. Izolacija filamentoznih plesni iz zemljišta i vazduha sa izrazitom sposobnošću stvaranja lakaza na specifičnim supstratima.
2. Ispitivanje najboljih sojeva plesni i zavisnosti njihove sposobnosti stvaranja lakaza od supstrata.
3. Identifikacija najboljih sojeva u odnosu na stvaranje lakaza.
4. Ispitivanje i određivanje supstrata pogodnih za stvaranje lakaza.
5. Ispitivanje aktivnosti rastvorenih lakaza u podlogama u odnosu na specifične zagađujuće materije.
6. Ispitivanje stabilnosti (“vremena poluživota”) lakaza u određenim tipovima voda i zemljišta.
7. Uporedno laboratorijsko ispitivanje aktivnosti lakaza plesni i njihovih smesa u odnosu na detoksikaciju određenih fenolnih i anilinskih derivate u vodi i zemljištu.
8. In field” ispitivanja aktivnosti lakaza u odnosu na 2,4-dihlorfenol i 3,4-dihloranilin u zemljištu sa određenim biljnim kulturama.

## Programme

### 31 August

8.00-9.30 Registration of the participants

9.30-9.35 Welcome speech, Prof. Dr. Csaba Vágvölgyi, Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Hungary

9.35-10.00 **Zsuzsanna László**, Zsolt Kiss, Sándor Beszédes, Cecília Hodúr: "*Elimination of phenols from water by AOP's and combined water treatment processes*", Institute of Process Engineering, Faculty of Engineering, Univer-sity of Szeged, Hungary

10.00-10.30 **András Papp**: *„Pesticide residues in the environment: sources of health risk",* Depart-ment of Public Health, Faculty of Medicine, University of Szeged, Hungary

*10.30-11.00 Coffee break*

11.00-11.30 **László Erdei**: „*Phytoremediation of heavy metal contaminated environment: from labora-tory to practice*", Department of Plant Biology, Faculty of Science and Informatics, University of Szeged, Hungary

11.30-12.00 Krisztián Laczi, Ágnes Kis, Kornél L. Kovács, **Gábor Rákhely**, Katalin Perei: „*Microbial tools for removal of unctuous pollutants*", Depart-ment of Biotechnology, Faculty of Science and Informatics, University of Szeged, Hungary

*12.00-13.00 Lunch break*

13.00-13.30 Botond Hegedűs, Mónika Magony, Krisztián Laczi, **Katalin Perei**, Gábor Rákhely: „*Metabolic pathways for biodegradation of sulfonated aromatic xenobiotics*“, Department of Biotechnology, Faculty of Science and Informatics, University of Szeged, Hungary

13.30-14.00 **Márta Huszka**, Renáta Rauch, Rita Földényi: „*Alginite as a sorbent of organic contaminants*“, Department of Earth and Environmental Sciences, University of Pannonia, Veszprém, Hungary

14.00-16.00 Laboratory visit at the Department of Microbiology, FSI, University of Szeged, Hungary

*16.00-16.30 Coffee break*

16.30-18.30 Round table discussion with workshop participants

**1 September**

10.00-13.00 “In field” visit to one representative of the Regional Cross-Border Bio-production Learning Network 1.

*13.00-14.00 Lunch break*

14.00-17.00 “In field” visit to one representative of the Regional Cross-Border Bio-production Learning Network 2.

## Program

### Augusztus 31.

8.00-9.30 Résztvevők regisztrációja

9.30-9.35 Nyitóbeszéd, Prof. Dr. Vágvölgyi Csaba, tanszékvezető egyetemi tanár, SZTE TTIK Mikrobiológiai Tanszék, Szeged, Magyarország

9.35-10.00 **László Zsuzsanna**, Kiss Zsolt, Beszédes Sándor, Hodúr Cecília: "*Fenolok eltávolítása vízből fejlett oxidációs módszerekkel (AOP) és kombinált vízkezelési eljárásokkal*", SZTE MK Folyamatmérnöki Intézet, Szeged, Magyarország

10.00-10.30 **Papp András**: *„Peszticidmaradványok a környezetben, mint egészségkockázati források",* SZTE ÁOK Népegészségtani Intézet, Szeged, Magyarország

*10.30-11.00 Kávészünet*

11.00-11.30 **Erdei László**: „*Nehézfémekkel szennyezett környezet fitoremediációja: a laboratóriumtól a gyakorlati alkalmazásokig*", SZTE TTIK Növénybiológiai Tanszék, Szeged, Magyarország

11.30-12.00 Laczi Krisztián, Kis Ágnes, Kovács L. Kornél, **Rákhely Gábor**, Perei Katalin: „*Mikrobiális eszközök olajos szennyezőanyagok eltávolítására*", SZTE TTIK Biotechnológiai Tanszék, Szeged, Magyarország

*12.00-13.00 Ebédszünet*

13.00-13.30 Hegedűs Botond, Magony Mónika, Laczi Krisztián, **Perei Katalin**, Rákhely Gábor: „*Metabolikus utak szulfonált aromás xenobiotikumok biodegradációjára*“, SZTE TTIK Biotechnológiai Tanszék, Szeged, Magyarország

13.30-14.00 **Huszka Márta**, Rauch Renáta, Földényi Rita: „*Az alginit, mint szerves szennyezőanyagok szorbense*“, Föld- és Környezettudományi Intézeti Tanszék, Pannon Egyetem, Veszprém, Magyarország

14.00-16.00 Laboratórium-látogatás a Szegedi Tudományegyetem Természettudományi és Informatikai Karának Mikrobiológiai Tanszékén

*16.00-16.30 Kávészünet*

16.30-18.30 Kerekasztal-beszélgetés a Workshop résztvevőivel

**Szeptember 1.**

10.00-13.00 “Tereplátogatás” a Regionális Határon Átnyúló Biotermesztési Tanulás-hálózat egyik képviselőjénél 1.

*13.00-14.00 Ebédszünet*

14.00-17.00 “Tereplátogatás” a Regionális Határon Átnyúló Biotermesztési Tanulás-hálózat egyik képviselőjénél 2.

## Programa

### 31. avgust

8.00-9.30 Registracija

9.30-9.35 Pozdravna reč - Prof. Dr. Csaba Vágvölgyi, Departman za mikrobiologiju, Fakultet prirodnih nauka, Univerzitet u Segedinu, Segedin, Mađarska

9.35-10.00 **Zsuzsanna László**, Zsolt Kiss, Sándor Beszédes, Cecília Hodúr: "*Uklanjanje fenola iz vode pomoću AOP i kombinovanih procesa obra-de*", Institut procesnog inženjeringa, Inženjer-ski fakultet, Univerzitet u Segedinu, Segedin, Mađarska

10.00-10.30 **András Papp**: *„Ostaci pesticida u životnoj sredini: rizik po ljudsko zdravlje",* Departman za javno zdravlje, Medicinski fakultet, Univerzitet u Segedinu, Segedin, Mađarska

*10.30-11.00 Kafe pauza*

11.00-11.30 **László Erdei**: „*Fitoremedijacija sredine zagađene teškim metalima: od laboratorije do prakse*", Departman za biologiju biljaka, Fakultet prirodnih nauka, Univerzitet u Segedinu, Segedin, Mađarska

11.30-12.00 Krisztián Laczi, Ágnes Kis, Kornél L. Kovács, **Gábor Rákhely**, Katalin Perei: „*Mikrobiološko uklanjanje zauljenih otpadnih materija*", Departman za biotehnologiju, Fakultet prirodnih nauka, Univerzitet u Segedinu, Segedin, Mađarska

*12.00-13.00 Pauza za ručak*

13.00-13.30 Botond Hegedűs, Mónika Magony, Krisztián Laczi, **Katalin Perei**, Gábor Rákhely: „*Metabolički putevi biorazgradnje sulfonovanih aromatičnih ksenobiotika*“, Departman za biotehnologiju, Fakultet prirodnih nauka, Univerzitet u Segedinu, Segedin, Mađarska

13.30-14.00 **Márta Huszka**, Renáta Rauch, Rita Földényi: „*Alginit kao sorbent organskih zagađujućih jedinjenja*“, Departman za nauku o Zemlji i životnoj sedini, Universitet Pannonia, Veszprém, Mađarska

14.00-16.00 Poseta laboratoriji Departmana za mikrobiologiju, Fakultet prirodnih nauka, Univerzitet u Segedinu, Segedin, Mađarska

*16.00-16.30 Kafe pauza*

16.30-18.30 Okrugli sto-diskusija sa učesnicima

**1. septembar**

10.00-13.00 “In field” poseta predstavniku Regional Cross-Border Bio-production Learning Network 1.

*13.00-14.00 Pauza za ručak*

14.00-17.00 “In field” poseta predstavniku Regional Cross-Border Bio-production Learning Network 2.

# ELIMINATION OF PHENOLS FROM WATER BY AOP'S AND COMBINED WATER TREATMENT PROCESESS

**László, Zs., Kiss, Zs. L., Beszédes, S., Hodúr, C.**
Institute of Process Engineering, Faculty of Engineering, University of Szeged, Hungary

Many organic compounds are resistant to conventional chemical and biological treatments. For this reason, other methods are being studied as alternatives to biological and classical physico-chemical processes. Of these, Advanced Oxidation Processes (AOP's) constitute one of the best options in the near future. AOP's are those aqueous phase oxidation processes which are based primarily on generation of the hydroxyl radical, which can react very fast with organic compounds, resulting in the destruction of the target pollutant. The AOP's are pollutant treatment processes, which use ozone, UV, VUV, ozone in combination with UV ($O_3$/UV), ozone plus hydrogen peroxide ($O_3$/$H_2O_2$), hydrogen peroxide and ultraviolet light (UV/$H_2O_2$), Fenton's reagent and heterogeneous photocatalysis which uses titanium dioxide ($TiO_2$) in combination with light (UV) and oxygen. The main problem of AOP's lies in the high cost of reagents such as ozone, hydrogen peroxide or light sources like ultraviolet or vacuum-ultraviolet light. These costs are increasing with the increasing volume of the water to be treated, thus in some cases it is more proper to concentrate the pollutants e.g. by membrane filtration processes, and to treat the retentate containing higher concentration of pollutants. Benefits of preconcentration are more perceptible considering that the kinetics seems to be first order with respect to hydroxyl radical concentration and to the pollutant, which means that the reaction rate is increasing with increasing pollutant concentration.

Membrane-based separation processes are proving to be promising alternatives for conventional industrial separation methods, since they offer numerous advantages, such as no chemical additives are needed to eliminate the pollutants, there is no disinfection by-product production. Although the membrane technique became a growing industry, the determination of relationships between operational parameters and development of models need to planning effective processes several problems need to be solved. Membrane fouling is the most important problem in membrane filtration practice, it is one of the most limiting factors for wider membrane applications causing significant flux decline, increased operating costs and decreased membrane lifetime, thus recent researches are focused on this area. Combining membrane separation processes with AOP's in water treatment offers new opportunities because ozone and hydroxyl radicals are able to decompose certain membrane foulants very efficiently.

Although phenol and phenolic compounds are relatively easily degradable by biological methods, the nature of the molecule makes it „ideal” test compound for investigation of AOP's. There are numbers of publications concerning degradation of phenol by AOP's. Comparing several AOP's (e.g. ozone treatment, VUV photolysis and heterogeneous photocatalysis) it has been found that in all systems almost the same aromatic and aliphatic intermediates are formed, and the change of the TOC follows similar trends in the function of phenol conversion. The similar byproduct-distribution indicates that the different advanced oxidation methods are similar in character and the degradation can be described in terms of a similar reaction pathway.

In the southern part of Hungary, there are number of thermal wells used for heating e.g. greenhouses. The residual thermal waters have high ion content and in some cases high organic content including phenolic compounds.

The aim of our work was the investigation of the elimination of phenol content from residual waste thermal waters by combination of membrane filtration and ozone treatment.

There are two ways to combine the AOP's with membrane separation. The preconcentration by membrane filtration makes the degradation of pollutants more effective. This method may be applicable in cases where the retentate contains high concentration of non-biodegradable pollutants. The result of AOP's treatment may be the total elimination of pollutants, or in other cases the partial degradation and transformation to more biodegradable form. It must be noted that the application of these technologies in discrete cases needs circumspection because the partial degradation of some pollutants may result in increased toxicity.

The effect of pre-ozonation on efficiency on membrane separation was also investigated in case of several types of waste waters. In this method the microflocculation effect of ozone on colloid solutions is utilized to coagulate and flocculate the micropollutants of waste water.

Examinations have been dealt to remove the phenol content of a model waste thermal water resulted that the ozone treatment degrades the phenol content and decrease the organic content of the residual thermal waste water. The preozonation increased the purification efficiency regarding both COD and ion content. The analysis of experimental results (membrane resistances, fouling and retention) showed that the appearance and diminishing of degradation by-products determine the efficiency of the nanofiltration probably by changing membrane-solution interactions.

The authors are grateful for the financial support provided by the project „Optimization of Cost Effective and Environmentally Friendly Procedures for Treatment of Regional Water Resource” - IPA Cross-border Co-operation Programme (Serbia-Hungary), HUSRB 0901.

## Curriculum vitae of Zsuzsanna László

Zsuzsanna László was born in 1973 in Szekszárd. She received PhD degree in the field of environmental chemistry in 2001, her research area was the investigation of VUV photolysis of organic pollutants of the water. She is currently an Associate Professor working in the Institute of Process Engineering at the Faculty of Engineering of the University of Szeged. She is teaching technical chemistry and environmental engineering techniques and processes. Her primary research interests include application of advanced oxidation processes in food industry focusing on water treatment, effect of AOP’s on biodegradability of organic pollutants and combination of AOP’s and membrane separation processes for removal of organic compounds from wastewater.

# PESTICIDE RESIDUES IN THE ENVIRONMENT: A SOURCE OF HEALTH RISK

**Papp, A.**
Department of Public Health, Faculty of Medicine, University of Szeged, Hungary

Agricultural production, both plant growing and animal husbandry, is inevitably a major intervention into the normal processes and development of the ecosystems. “Useful” species are cultivated and propagated while others, regarded unwanted or directly “harmful”, are suppressed or eliminated. All that to the benefit of the human race, itself living in vastly higher abundance than it would be possible under “natural” (here: prehistoric) conditions.
The protection of crops from various pests - insects, rodents, moulds etc. - has had a long history from primitive means to the modern, chemistry-based pest control. However, the negative aspects of exclusive and indiscriminate use of chemical pesticides became more and more evident during the last 5-6 decades. The purpose of pesticides is, namely, to kill or incapacitate certain groups of living organisms; which simply means that pesticides are toxic agents, deliberately brought into those parts of the environment where humans get their food from.
Pesticides are supposed to act only on the pest to be eliminated, the target species, and within the intended limits of location and time; but such a sharp calculation of effect seldom works in reality. First of all, the selectivity of pesticide agents is frequently bad. This may be due, in case of insecticides, to phylogenetic similarity of the agents’ site of action in the target organism, typically an insect, and in non-target species including humans. This is the case e.g. with neurotoxic agents acting on ion channels. Effects dissimilar to the intended one, such as endocrine disruptor

effect of a neurotoxic agent or a plant hormone analogue, are also known.

Another property determining the unwanted effects of pesticides in the environment is their persistence. Today, pesticide agents of longer persistence (thus falling in the category of persistent organic pollutants) are undesired and their use has mostly been banned.

The general public, the consumers, can be exposed to environmental residues of pesticides via food or via drinking water. A number of such cases have been reported, indicating the real importance of the problem. In the lecture, some of such cases are presented in more detail.

## Curriculum vitae of András Papp

Dr. András Papp was born on 9 December 1957 in Budapest. He studied biology at the Faculty of Science of the József Attila University, Szeged (now: University of Szeged) and obtained his degree in 1982. Between 1982 and 1990 he worked at the Department of Comparative Physiology in Szeged and studied the cellular mechanisms of epileptic activity. In 1990 he received a 3-year fellowship at the Max Planck Institute of Brain Research in Frankfurt am Main, Germany. From 1993 on he has been at the Department of Public Health, Faculty of Medicine, University of Szeged, and has been investigating the nervous system effects of environmental xenobiotics in animal experiments. He obtained PhD in 1995, and “Dr. habil.“ title in 2000. He has had two postgraduate students who successfully finished their PhD period.

# PHYTOREMEDIATION OF HEAVY METAL CONTAMINATED ENVIRONMENT: FROM LABORATORY TO PRACTICE

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In one of our previous projects a complex phyto(bio)-remediation technology was worked out for the removal of heavy metals (HM) from the contaminated environment and for the safe handling of the harvested plant material. To reach our aims, both basic and applied research was carried out. First of all the effects of HMs $Cu^{2+}$ and $Cd^{2+}$ on the antioxidant defence mechanism were determined in *Brassica juncea*. For the signalization, it was found that nitric oxide (NO) transient was involved in the $Cu^{2+}$-induced pathway while $Cd^{2+}$ did not evoke NO-response. Both for basic and applied point of view it was important to work out a biosensor construction using the transgenic cyano-bacterium *Synechocystis* which well responded to the presence of $Ni^{2+}$, $Zn^{2+}$, $Co^{2+}$, $Cd^{2+}$, $Cr^{6+}$ and $As^{3+}$ ions.
In the contaminated soil, indigenous bacterium species contribute to mobilization or chelation of metal ions and often they have a positive effect on root development. We have isolated bacterial consortium from the rhizosphere of willow grown on the contaminated soil, and found that they significantly promoted rooting of willow cuttings. The effects of different phytoextraction treatments to the HM content of moderately contaminated soils were also examined in field experiments on plots formed near the Tisza river.
The contaminated biomass was incinerated in a special, technically improved incinerator and from the ash HM concentrations were determined and recovery was calculated.

As a summary, the following technology was worked out:

- Monitoring of contaminated soil, determination of HM composition, balance of matter, time frame of remediation;
- Establishment of a plantation of HM accumulator species;
- Stimulation of metal uptake and root growth, monitoring of actual situation;
- Harvesting of the contaminated plant material;
- Special combustion technology for the plant material;
- Verification: monitoring, determination of HM concentration and content, balance calculation.

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## Curriculum vitae of László Erdei

Prof. dr. László Erdei was born in 1945 in Törtel, Hungary. He has got his diploma at the Eötvös Loránd University in 1969. He became doctor of biological sciences in 1989. Since 1995 he is professor in the Department of Plant Physiology, University of Szeged. He worked in the Netherlands, Japan, and Kuwait. He has got several awards and member of several research societies. Research areas of his interest are: membrane ATPase activity and K, Na transport in unicellular green algae; membrane structure and carrier-mediated ion transport through bimolecular lipid films and liposomes; ATPase activity and ion transport at different levels of organization, the mechanism of salt tolerance; regulation of ion transport in agriculturally important crops; feedback control, hormonal regulation; the mechanism of adaptation to drought and salt stresses in plants; oxidative stress; signal-response.

# MICROBIAL TOOLS FOR REMOVAL OF UNCTUOUS POLLUTANTS

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Industrial pollution represents one of the major problems for the world. Although, recent technologies tend to reduce the emission of hazardous substances; nevertheless environmental pollution still reaches high levels. There are many toxic compounds of industrial wastes which must be neutralized. Biological approaches using microorganisms to convert polluting materials are environmentally and economically sound tools for cleaning our environment.
Microorganisms are key players in biological removal of pollutants. Depending on the physico-chemical properties of the compounds, bioaugmentation and/or biostimulation can be used to enhance the biodegradation processes. Crude oil contaminations represent one of the most serious environmental problems in the world.
However, large amount of other unctuous pollutants are also emitted by food industry and commercial life. Therefore, their removal is in the primary focus of environmental sciences. However, strains capable to utilize hydrocarbons appear at the contaminated sites, but their metabolic activities are often restricted by the lack of other nutrients. Here we demonstrate that – in certain cases – a natural strain or microbial community can be exploited for bioremediation of oil contaminated soils. In our laboratory, a *Rhodococcus* sp. was isolated from hydrocarbon polluted sites and it was proven that the bacterium could efficiently degrade crude, diesel and heavy oils. Moreover, the strain could tolerate low temperature and certain salt concentrations therefore it

might be applied in oil mineralization after marine catastrophes.

According to the beneficial properties of our strain in diesel oil degradation, it might be successfully used in the cleanup of food industrial wastewaters. In this study, our aim was to demonstrate the applicability of this strain in bioremediation of food industrial and municipal wastes.

Lard, pig and poultry fat and cooking oil were used as sole carbon sources in minimal medium. The substrate utilization was demonstrated by measuring substrate level in the medium, the respiration activity and $CO_2$ production of the *Rhodococcus sp.* The strain consumed the available oxygen and released remarkable amount of carbon dioxide within a week, which means the bacterium can oxidize these materials. In addition, measurements of substrate concentration coincide with these data. Consequently, this strain is a promising waste cleaner in both oil and food industrial as well as housekeeping applications.

## Curiculum vitae of Gábor Rákhely

Gábor Rákhely was born in 1960 in Hungary. He got his chemist diploma at the József Attila University (Szeged, Hungary) in 1985. He worked at various departments and institutes of the university and the Biological Research Center. Since 1999 he works in the Department of Biotechnology and now he is the acting head of the department. He has got his PhD degree in 1997. His research areas are: redox metalloenymes, hydrogenase enzymes, hydrogen metabolism; microbial photosynthesis, enzymology and physiology of thermophilic prokaryote strains, processes in bioenergetics, regulation of gene expression, bioinformatics, molecular basics of microbial bioconversions, oxygenases, biocatalysis.

# METABOLIC PATHWAYS FOR BIODEGRADATION OF SULFONATED AROMATIC XENOBIOTICS

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*Novosphingobium subarcticum SA1* (*Sphingomonas subarctica* SA1, formerly identified as *P. paucimobilis*) alone is able to degrade sulfanilic acid. It was shown that the strain could catabolize five analogue aromatic compounds including protocatechuate, p-aminobenzoicacid and 4-hydroxybenzoate. The protein patterns of the strains grown on sulfonated and nonsulfonated molecules were distinct indicating alternative routes for the assimilation of these compounds. The genome of the strain was sequenced by new generation genome sequencers and revealed numerous genes of enzymes potentially catalyzing the oxidation of aromatic compounds. A genomic region containing the genes coding for sulfocatechol and protocatechuate dioxygenase were identified in distinct gene clusters. Upstream of the genomic region harboring the sulfocatechol dioxygenase (*scaD, scaE*) genes, other genes coding for sulfocatechol dioxygenase, sulfomuconate cycloisomerase (*scaA*), sulfomuconolactone hydrolase (*sca*B), oxidoreductase (*scaC*), permease (*orf*1) could be found in a single gene cluster. The analysis of enzymatic activity of cells grown on sulfo- or protocatechuate revealed, that the protocatechol and sulfocatechol pathways overlapped at the ring cleaving reaction, but the next step required different specific cycloisomerase enzymes.
Investigation of the proteins appearing upon sulfanilic acid induction disclosed proteins likely involved in the sulfanilic acid transport, conversion as well as the iron transport. The oxidation of sulfanilic acid requires iron containing enzymes,

therefore this would be reasonable that the increased iron demand of the dioxygenase enzymes would be provided by the induction of an iron transport pathway.

A whole genome transcript analysis was performed to disclose already discovered and so far unknown components of sulfanilic acid assimilation. This approach confirmed the earlier findings and resulted new components likely involved in nitrogen, sulphur metabolism and the transport mechanisms.

A localization study of the proteins likely involved in the transport of amino group and ring hydroxylation pointed out that these enzymes were membrane associated and they appeared simultaneously. Recombinant enzymes were produced in homologous host and the proteins copurifying with either of these proteins were identified. The results suggest a membrane-associated complex which is responsible for coupled uptake and conversion of sulfanilic acid.

## Curriculum vitae of Katalin Perei

Katalin Perei was born in 1964 in Hungary. She received her PhD degree in biology in 2001. She works at the Department of Biotechnology, Faculty of Science and Informatics, University of Szeged. Her research areas are: bioremediation, biodegradation, investigation of microbial degradation pathways.

# ALGINITE AS A SORBENT OF ORGANIC CONTAMINANTS

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Alginite is a special oil shale. Its organic material content is 5 - 50 % that is mostly kerogene. Static equilibrium experiments were carried out when various organic contaminants (pesticides, surfactants) were used as solutes and the alginite as sorbent. It was found that these compounds showed high retention in this oil shale. Since alginite contains different microorganisms (bacteria, residues of algae etc.), the biological degradation of these compounds can take place, too. The decay of certain type of pesticides like that of the chloroacetanilide-type herbicides was rather fast while one sulfonylurea-type (methsulfuron methyl) herbicide proved to be stable. Making a comparison among the degradation in the presence of alginite and in that of different soils it was found that the most investigated compounds decomposed significantly faster in the case of the oil shale than in the case of any of the soils.

## Curriculum vitae of Márta Huszka

Márta Huszka was born on 6 February in Kecskemét. She studies environmental engineering (from 2009) and technical management (from 2012) at University of Pannonia, Veszprém, Hungary. Her research area is the application of alginite for removing environmental pollutants and the investigation of growing and processing conditions of microalgae for energy production. In 2010 she took part at the final of the Freshhh 2010 on-line competition. She speaks English and German. She is an internal auditor conform to ISO 9000.

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